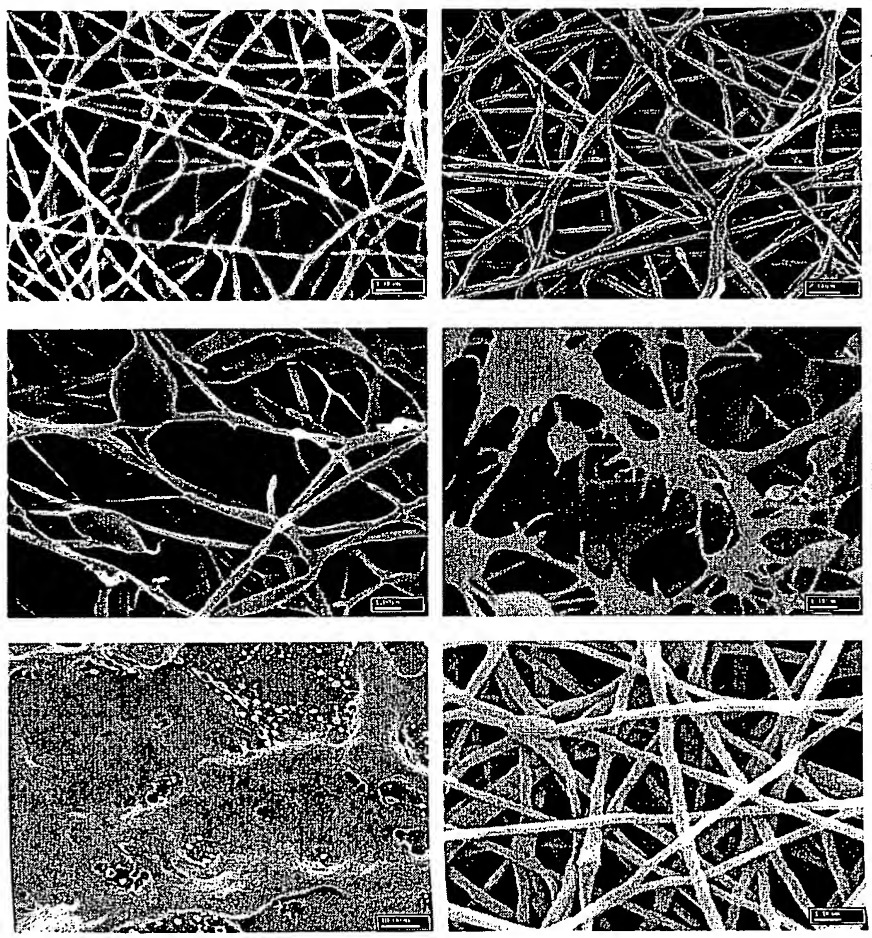


FIG. 1

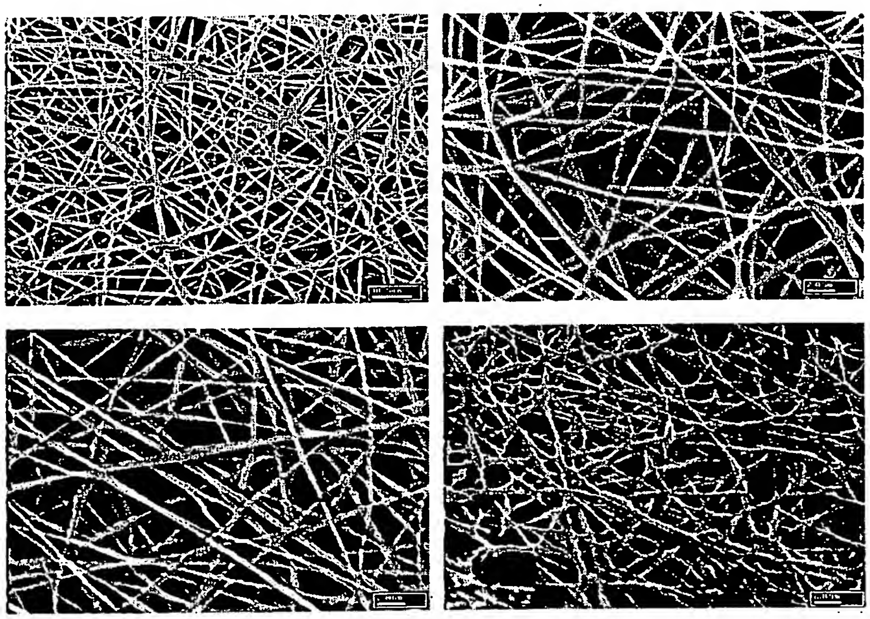


FIG. 2

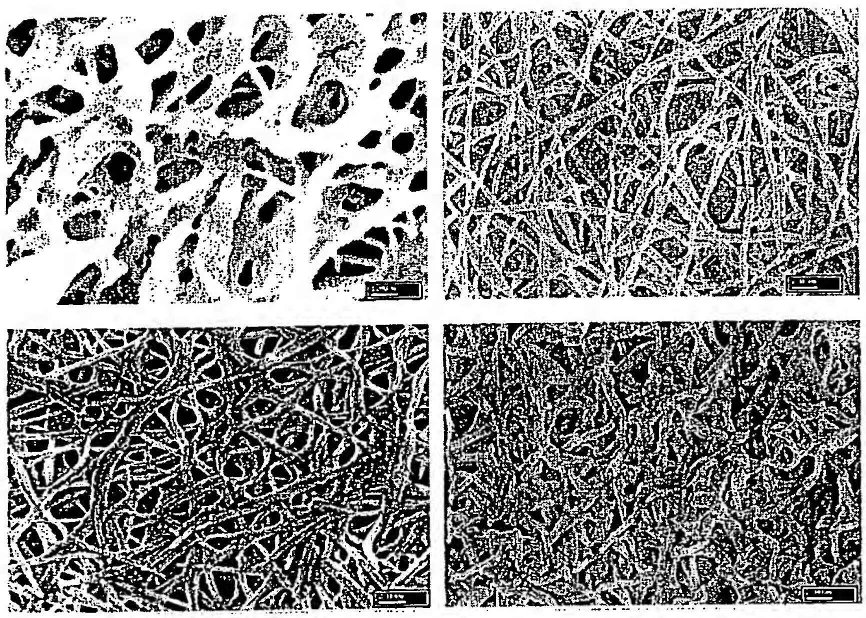


FIG. 3

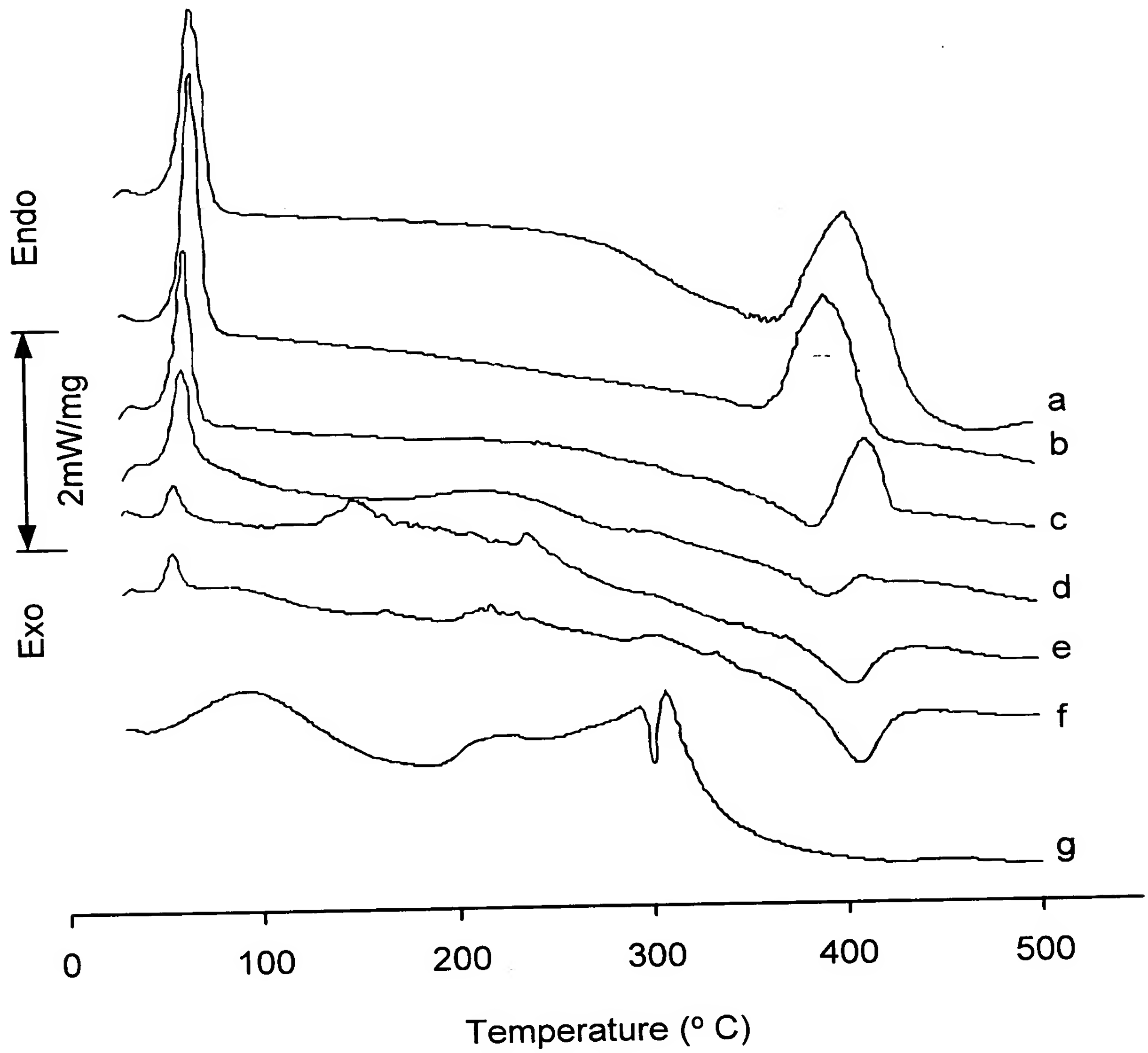


FIG. 4

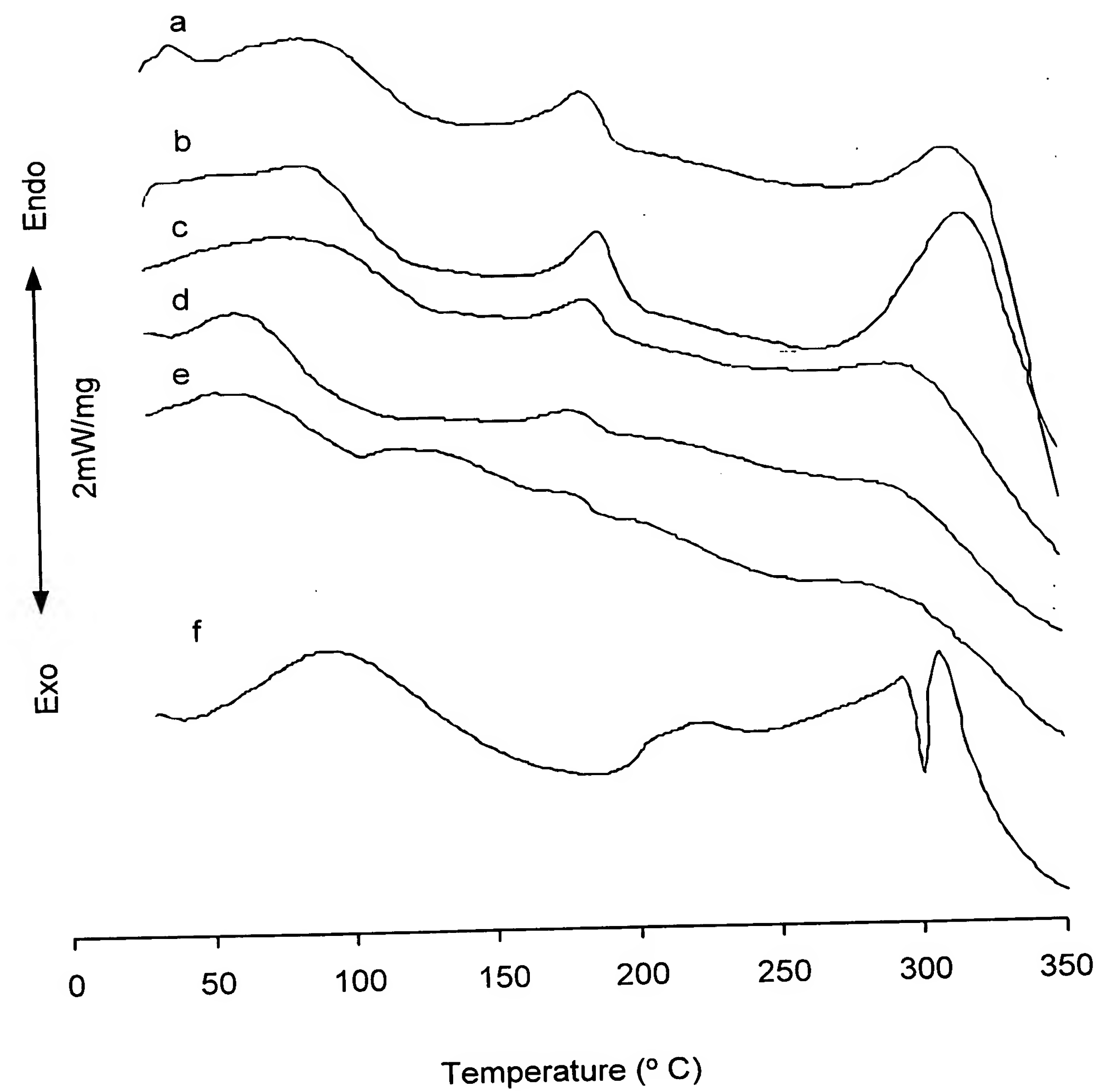

a
b
c
d
e
f
Endo
Exo
2mW/mg
0
50
100
150
200
250
300
350
Temperature (° C)

FIG. 5

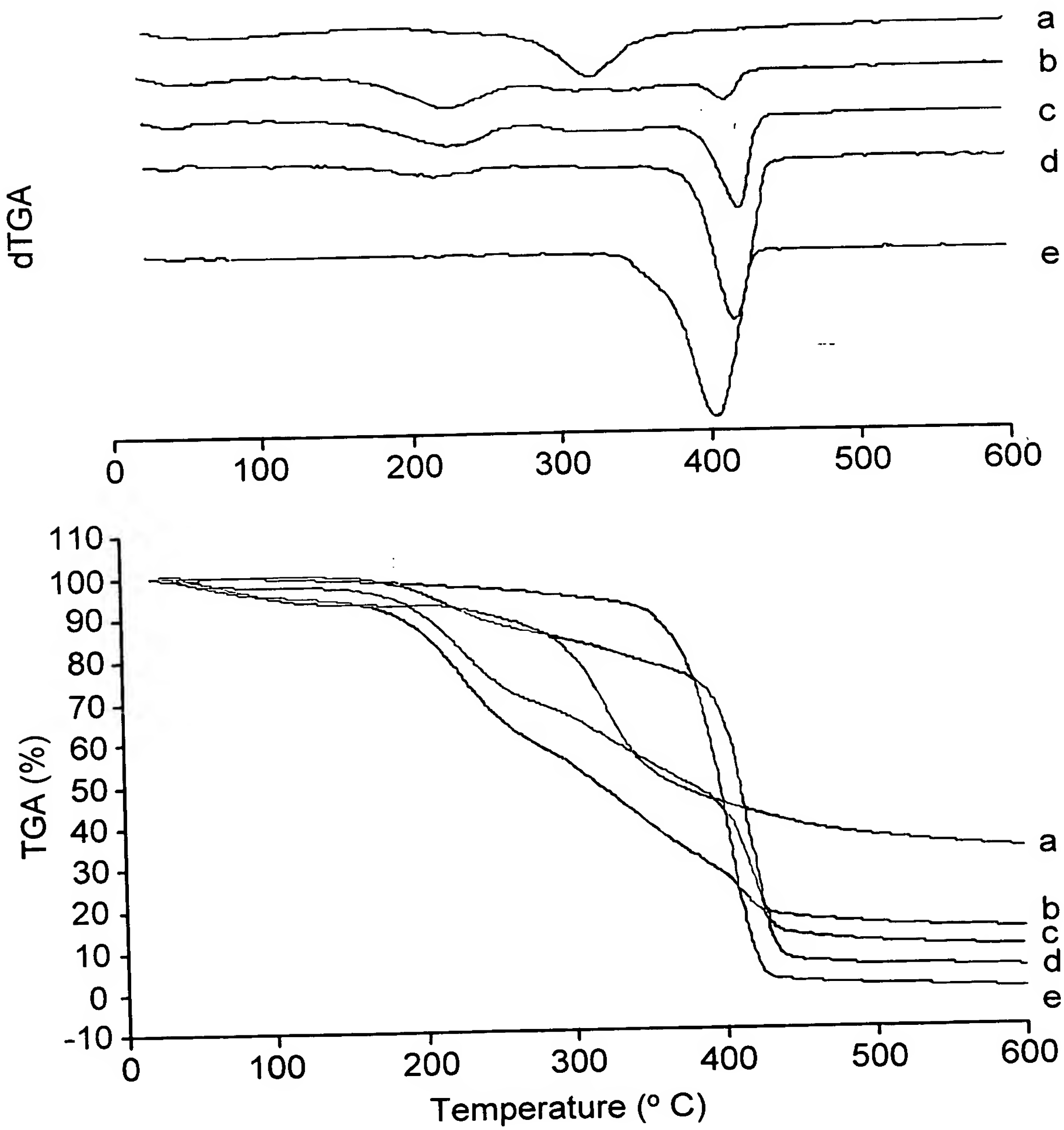

a
b
c
d
e
dTGA
0
100
200
300
400
500
600
110
100
90
80
70
60
50
40
30
20
10
0
-10
TGA (%)
a
b
c
d
e
0
100
200
300
400
500
600
Temperature (° C)

FIG. 6

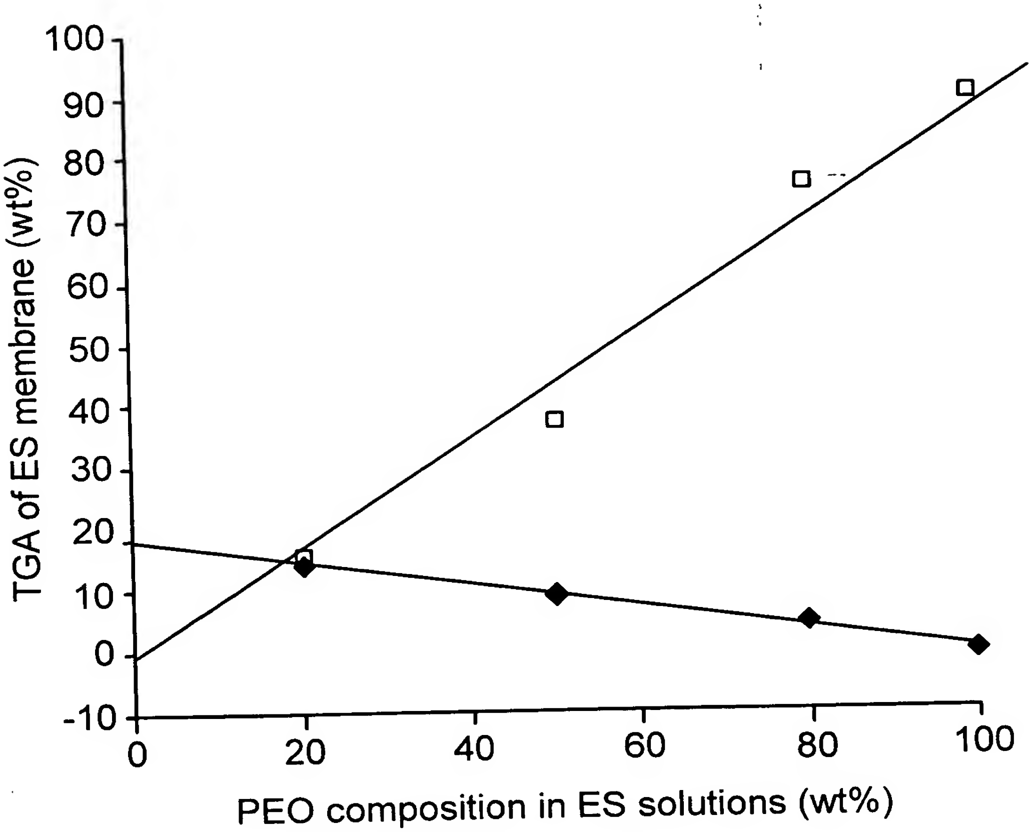

100
90
80
70
60
50
40
30
20
10
0
-10
TGA of ES membrane (wt%)
0
20
40
60
80
100
PEO composition in ES solutions (wt%)

FIG. 7

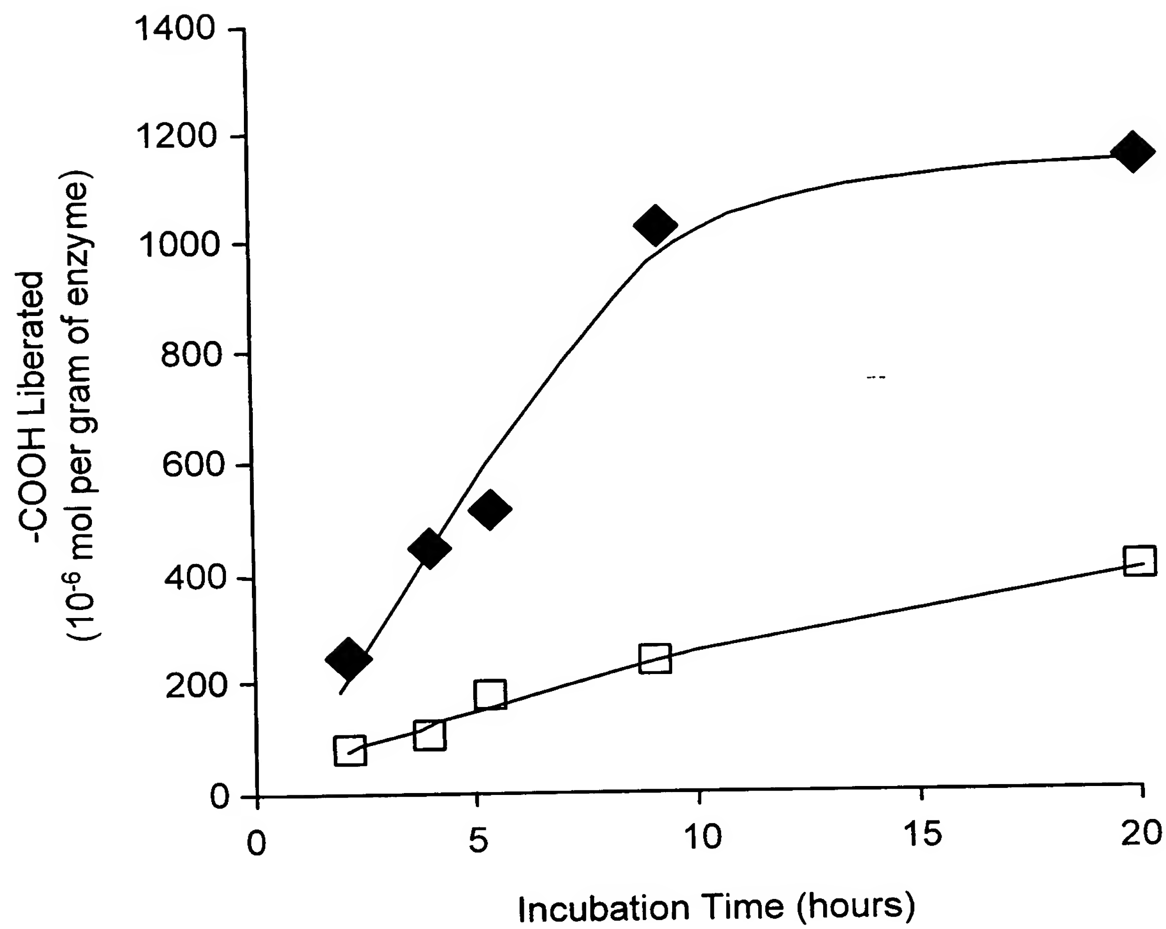

1400
1200
1000
800
600
400
200
0
-COOH Liberated
(10^{-6} mol per gram of enzyme)
0
5
10
15
20
Incubation Time (hours)

FIG. 8

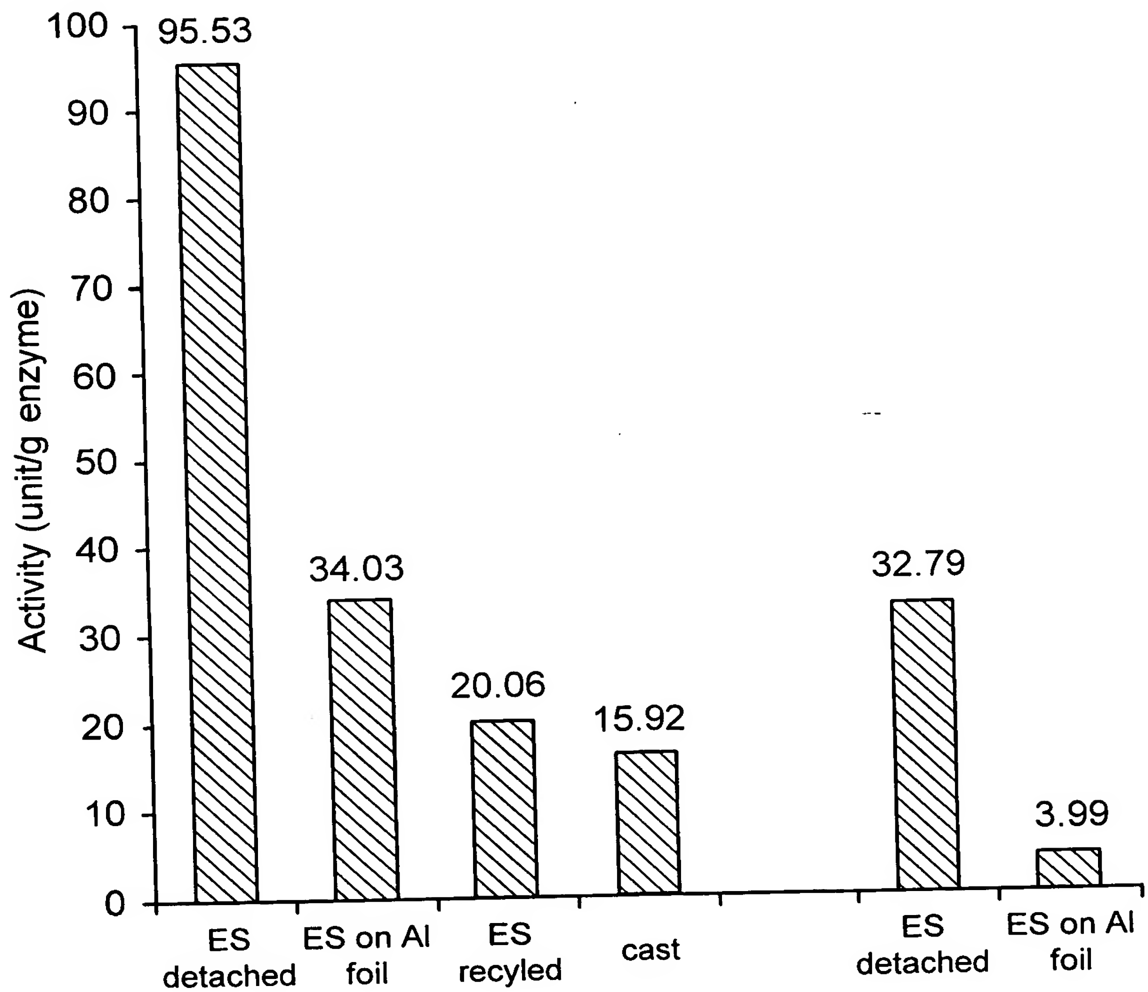

Activity (unit/g enzyme)
100
90
80
70
60
50
40
30
20
10
0
95.53
34.03
20.06
15.92
32.79
3.99
ES detached
ES on Al foil
ES recyled
cast
ES detached
ES on Al foil

FIG. 9

Reactive Groups on Proteins

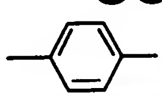
Reactive group	Amino Acid	pKa
$-NH_2$	Lysine(ε-NH_2),N-terminal amino groups (α-NH_2)	10.53;9.0-9.9
-COOH	Asparate,Glutamate,C-terminal carboxyl group	3.86;4.07;1.8-2.4
-SH	Cysteine	8.27
-SS-	Cystine	---
	Tyrosine	10.07

FIG. 10A

Reactions with Protein Amine NH_2

Reactive groups		Coupling Reaction
Acid anhydride	$(CO)_2O$	Peptide formation
Isocyanate	NCO	Peptide formation
Acylchloride	COCl	Peptide formation
Oxirane	$OCHXCH_2$	Alkylation
Aldehdye	CHO	Schiff base

FIG. 10B

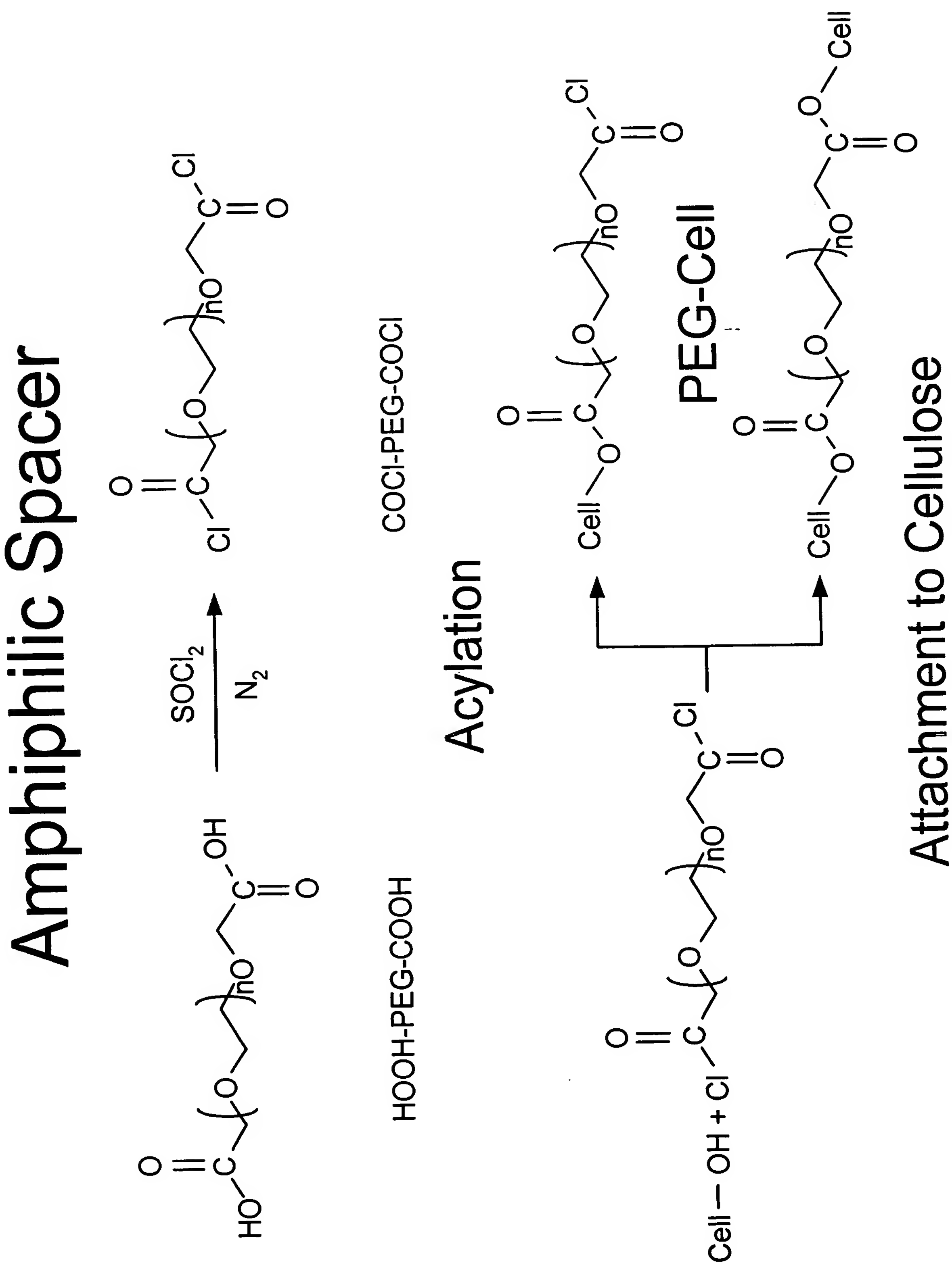

Amphiphilic Spacer
SOCl$_2$
N$_2$
HOOH-PEG-COOH
COCl-PEG-COCl
Acylation
Cell — OH + Cl
PEG-Cell
Attachment to Cellulose

FIG. 11

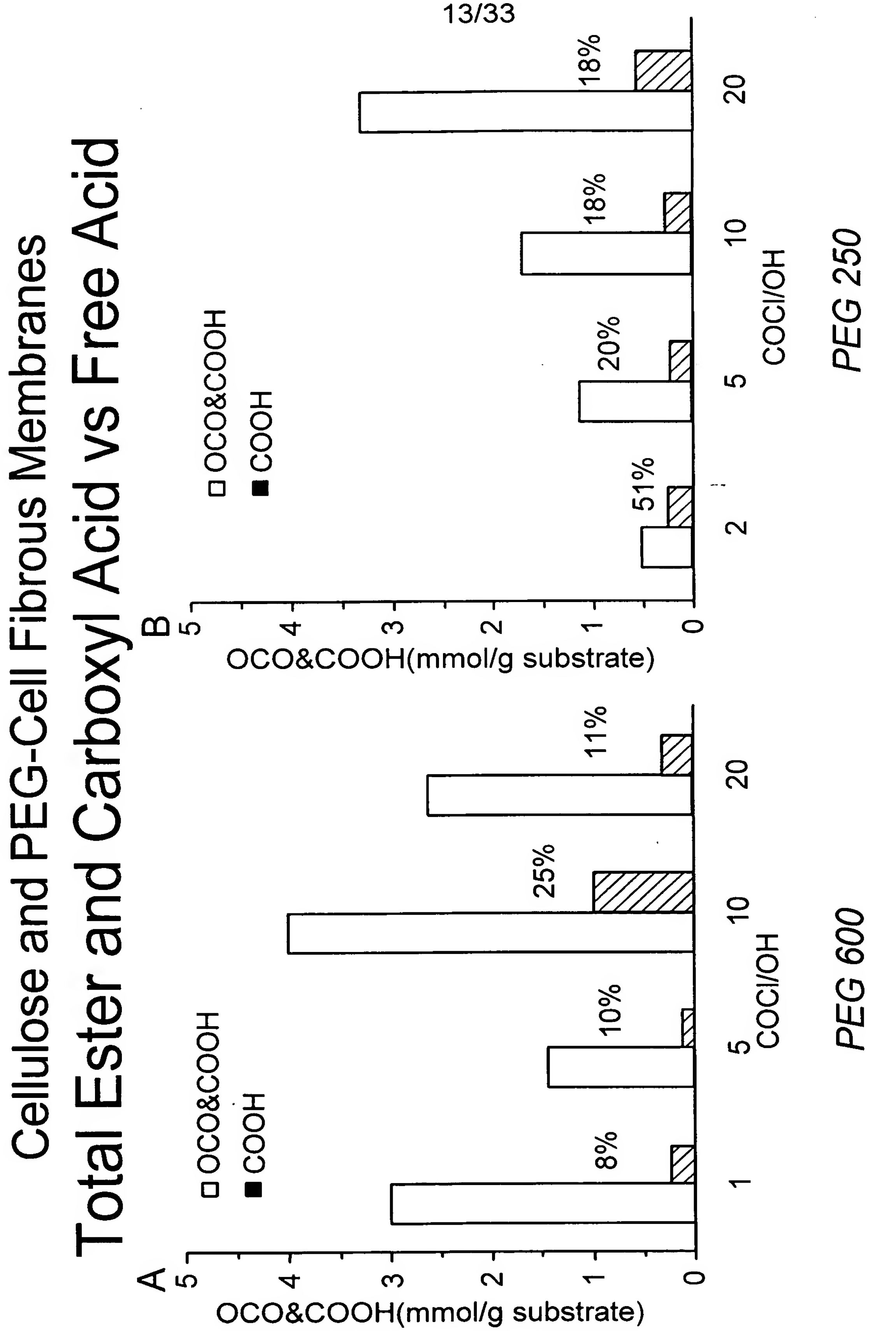
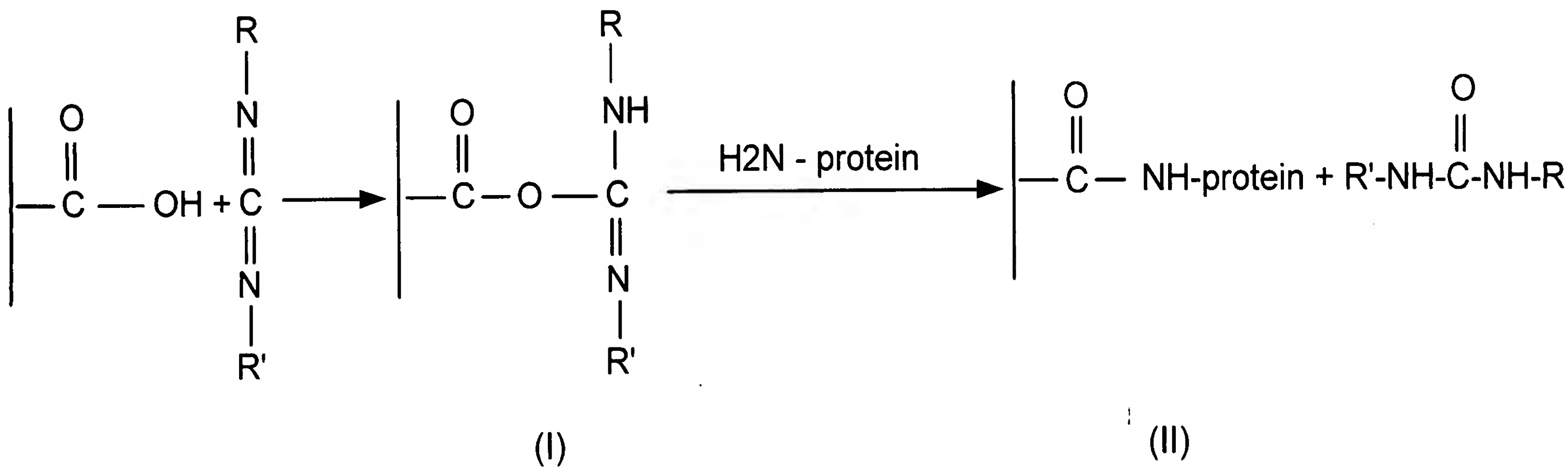

Cellulose and PEG-Cell Fibrous Membranes
Total Ester and Carboxyl Acid vs Free Acid
A
OCO&COOH
COOH
OCO&COOH(mmol/g substrate)
0
1
2
3
4
5
1
5
10
20
COCl/OH
8%
10%
25%
11%
PEG 600
B
OCO&COOH
COOH
OCO&COOH(mmol/g substrate)
0
1
2
3
4
5
2
5
10
20
COCl/OH
51%
20%
18%
18%
PEG 250

FIG. 12

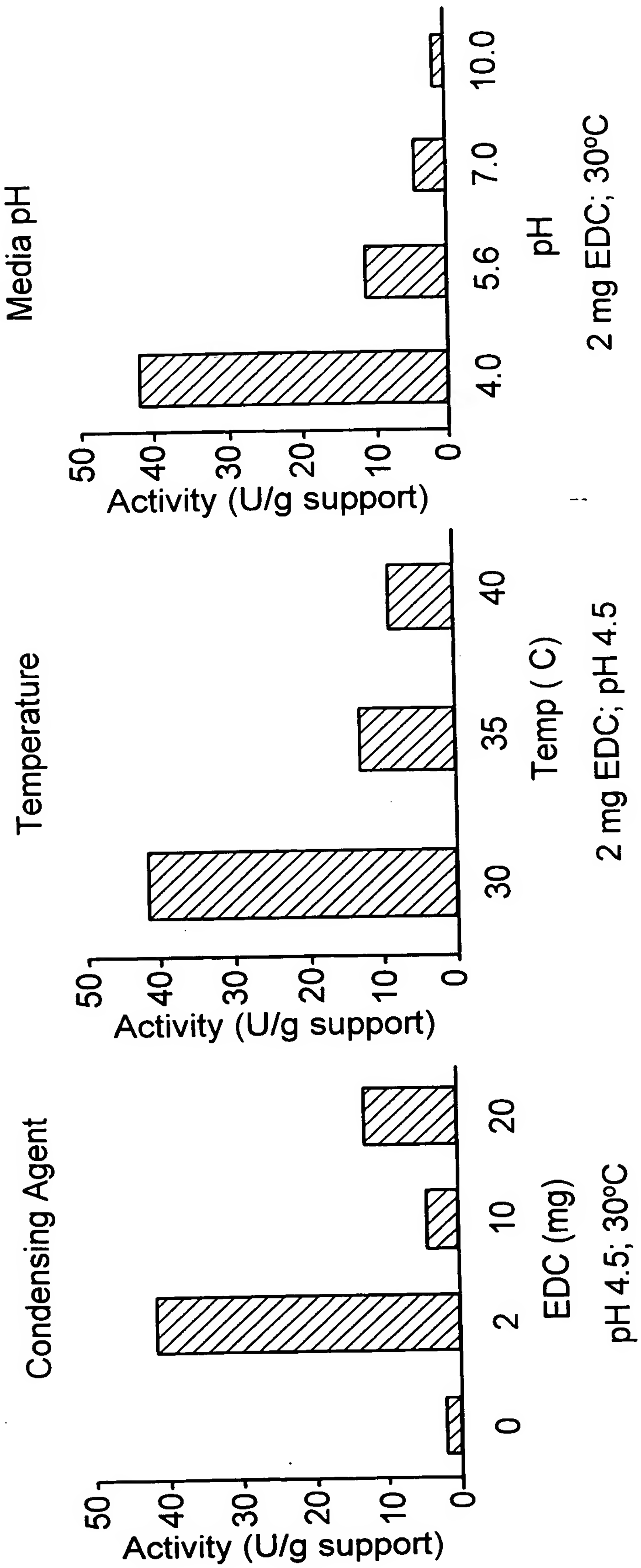
Coupling of Protein Amine and PEG-Cell Carboxylic via Carbodiimide



R, R' = CH_2CH_3, $CH_2CH_2CH_2\text{-}N(CH_3)_2$

FIG. 13

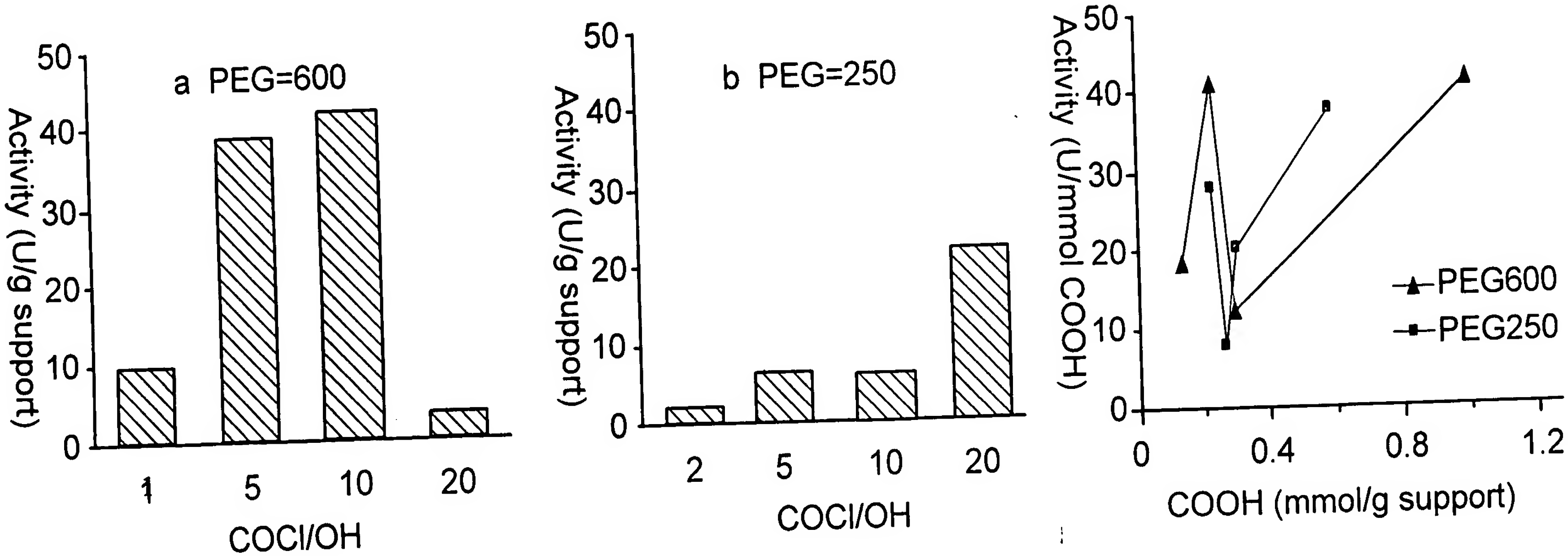
Lipase-PEG-Cell Fibrous Membranes -Coupling Reaction Conditions-



50 mg PEG-CELL support (PEG 600, 10 COCl/OH); 5 mg lipase;
5 ml aqueous buffer; 7 h.

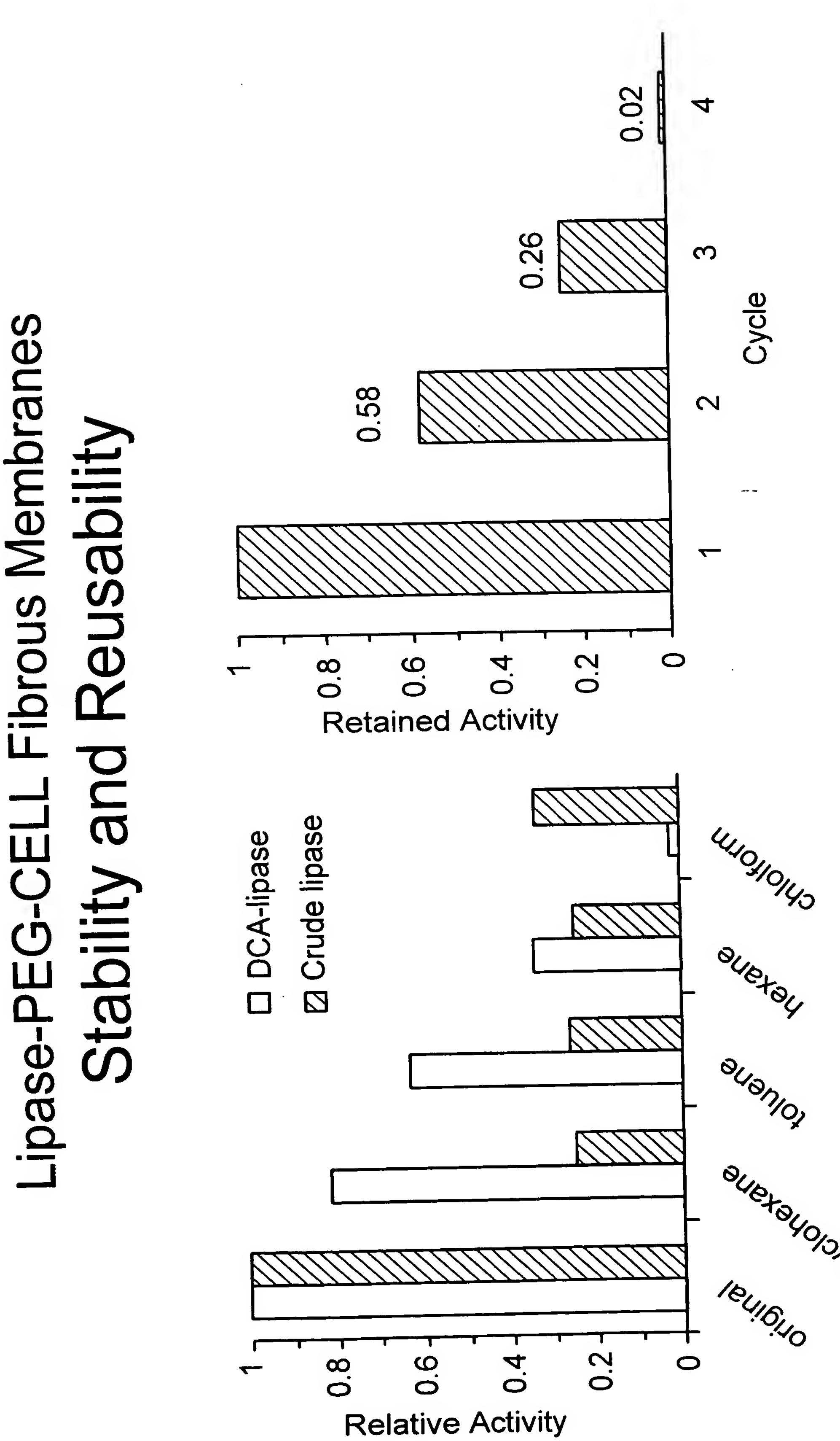
FIG. 14

Lipase-PEG-Cell Fibrous Membranes -varying COCl/OH ratios and PEG lengths-



50 mg PEG-CELL support; 5 mg lipase; 2 mg EDC;
5 ml aqueous buffer (pH 4); 7 h, 30°C.

FIG. 15

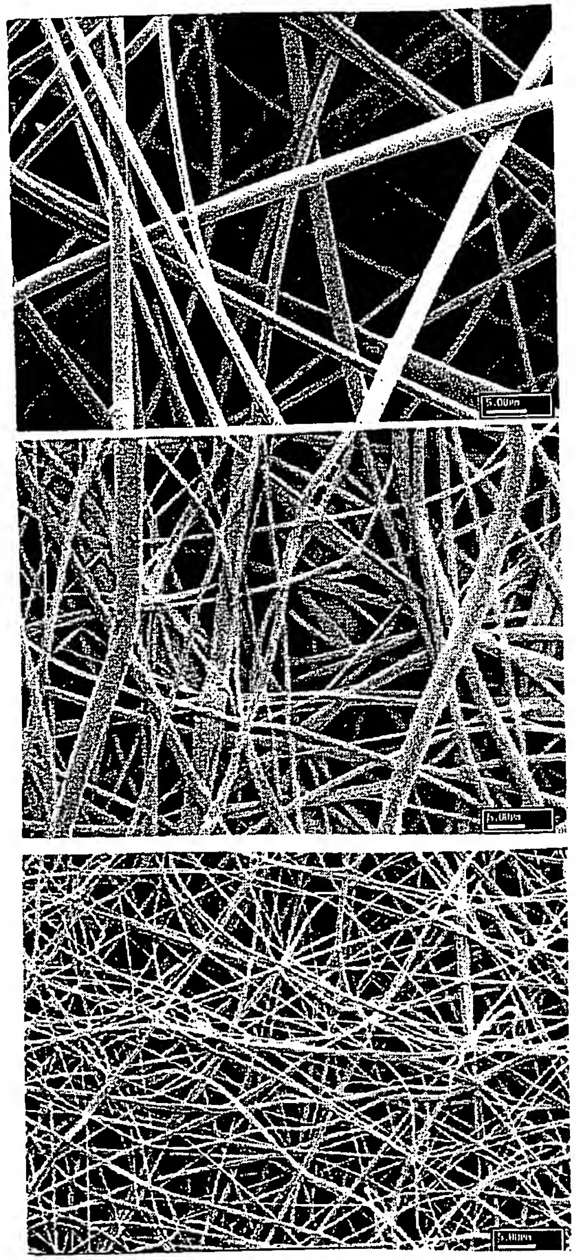


50 mg PEG-Cell support (PEG600, 10 COCl/OH); 5 mg lipase; 2 mg EDC; 5 ml pH 4 buffer; 7 h, 30°C.

50 mg PEG-Cell support (PEG250, 20 COCl/OH); 5 mg lipase; 2 mg EDC; 5 ml pH 4 buffer; 7 h, 30°C)

FIG. 16

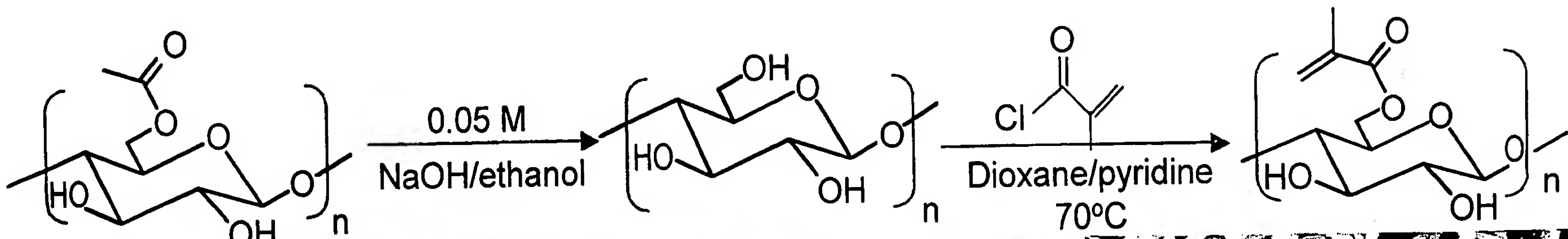
ES Cellulose Acetate
DS=2.45, 30,000Dalton
2:1 Acetone/DMAc

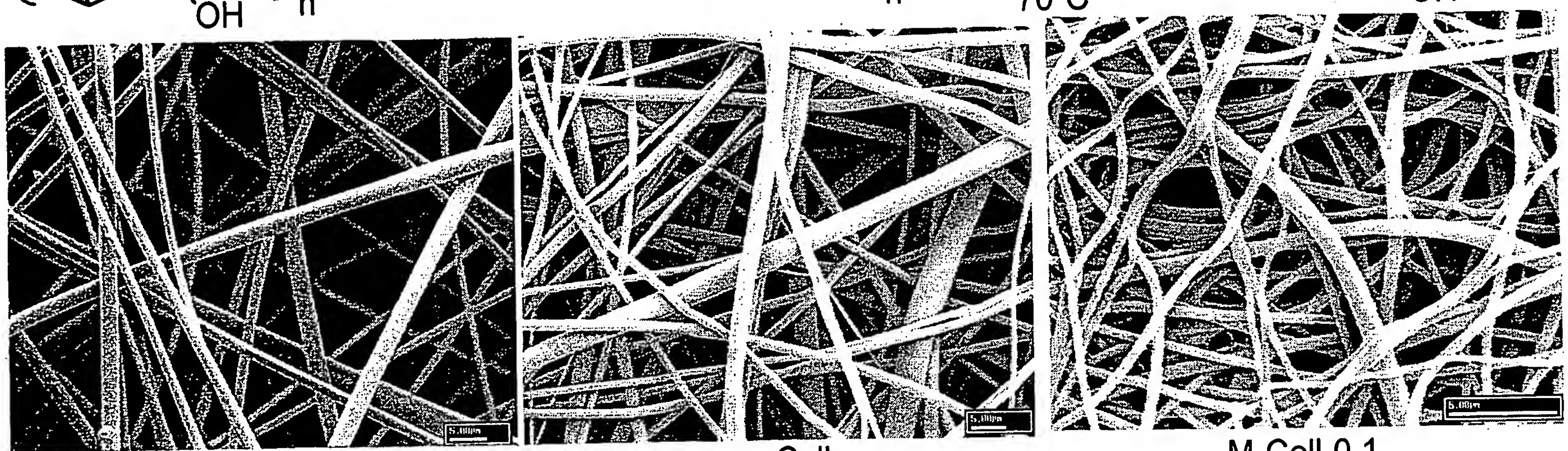


Target	Conc.	Porosity ϕ	Fiber Diameter nm	Pore Volume Total C_m ul/mg	Pore Volume Planar C_v ul.mg	C_v/C_m
Paper	20%CA	0.95	$\leq$3000	24.8	17.0	0.69
Water	20%CA	0.76	500-3000	6.3	3.1	0.49
Water	15%CA	0.43	100-500	3.0	1.0	0.33

FIG. 17

Ultra-fine Cellulose Fibers
Hydrolysis and Methacrylation





CA | Cell | M-Cell-0.1

	CA	Cell	M-Cell-0.1
θ_{H2O} ( )	84	56	84
C_m (ul/mg)	17.0	5.2	4.9
C_{H2O} (ul/mg)	0	13.0	4.3

FIG. 18

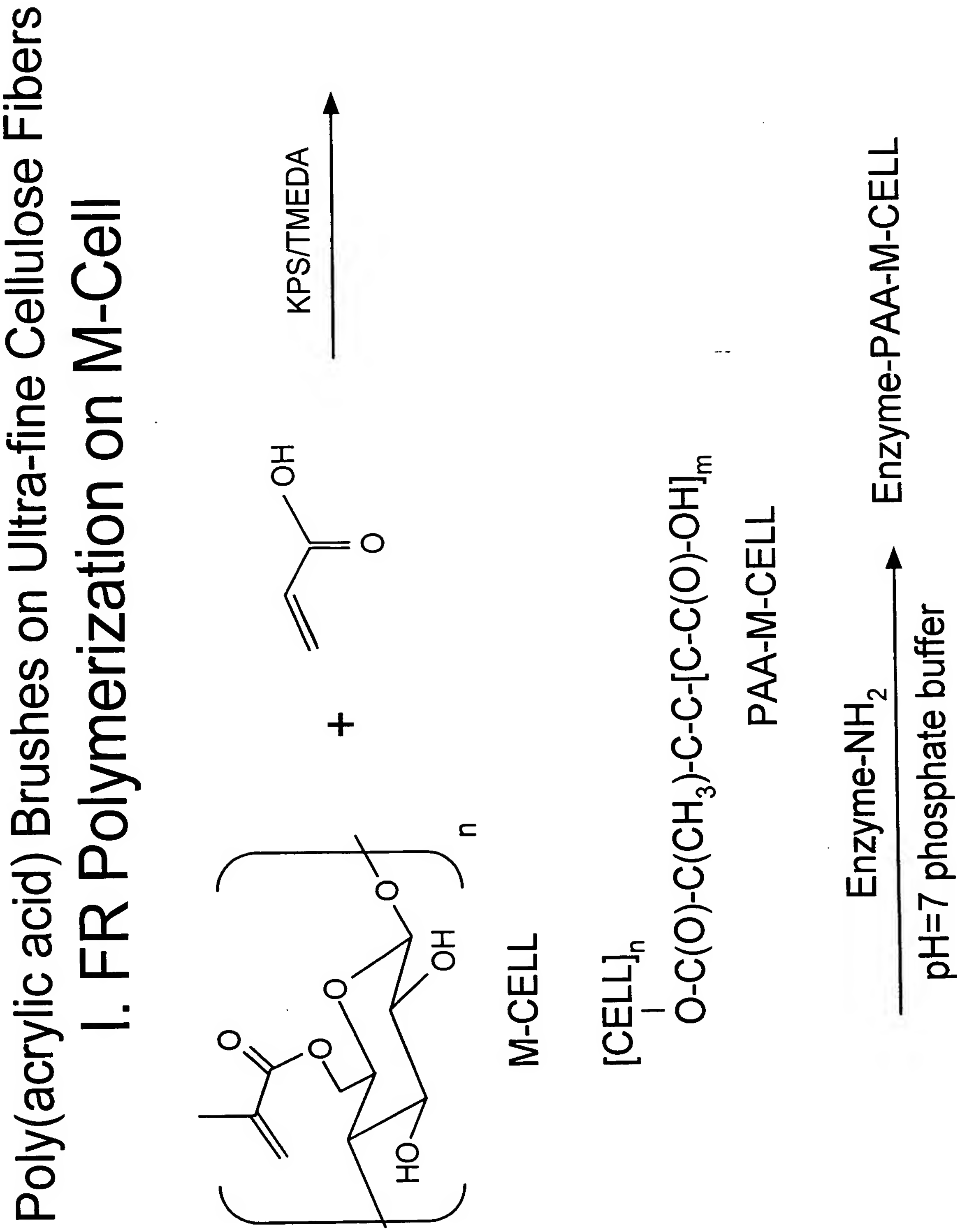

Poly(acrylic acid) Brushes on Ultra-fine Cellulose Fibers
I. FR Polymerization on M-Cell
KPS/TMEDA
M-CELL
[CELL]n
O-C(O)-C(CH3)-C-C-[C-C(O)-OH]m
PAA-M-CELL
Enzyme-NH2
pH=7 phosphate buffer
Enzyme-PAA-M-CELL

FIG. 19

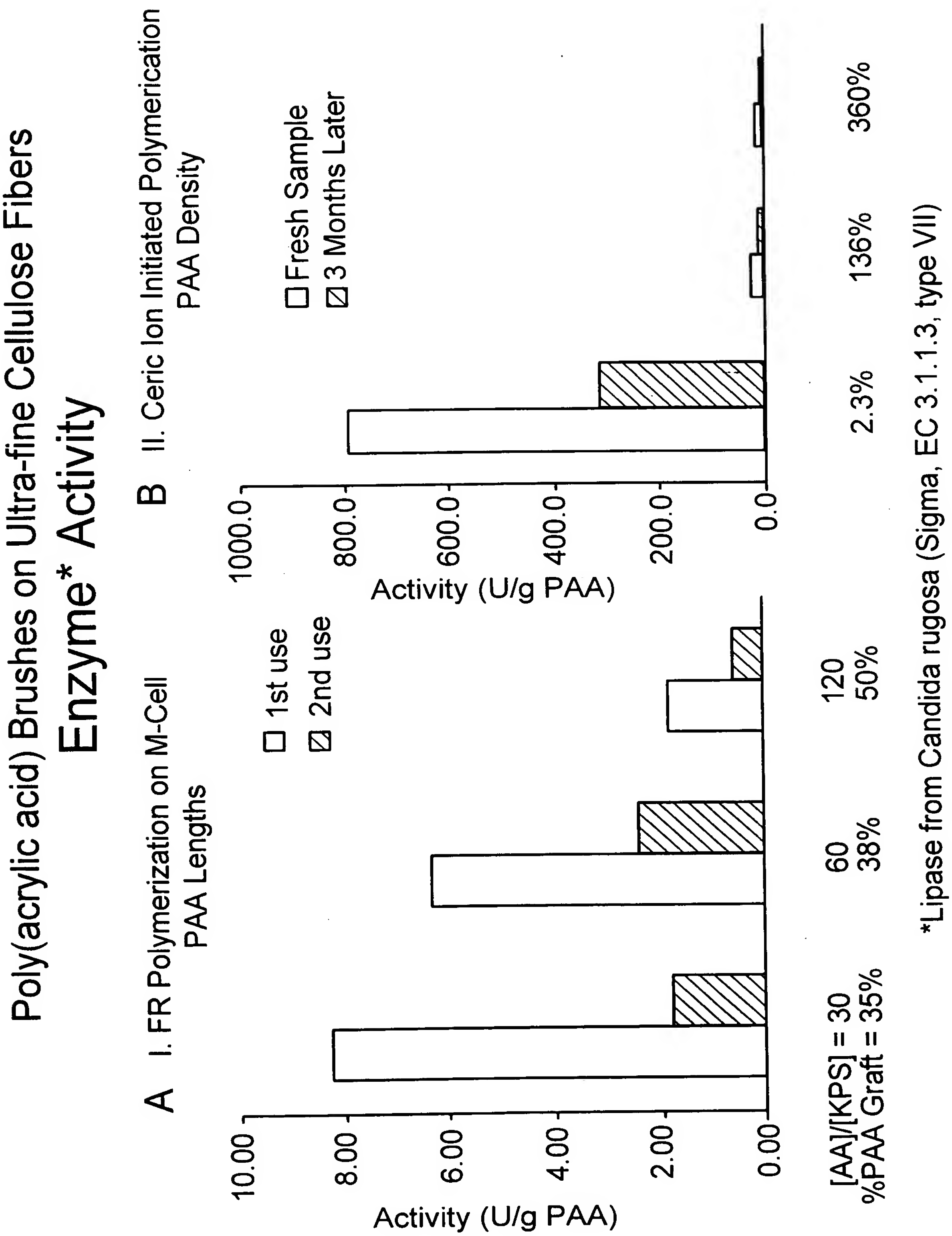

Poly(acrylic acid) Brushes on Ultra-fine Cellulose Fibers
Enzyme* Activity
A I. FR Polymerization on M-Cell
PAA Lengths
1st use
2nd use
Activity (U/g PAA)
10.00
8.00
6.00
4.00
2.00
0.00
[AA]/[KPS] = 30
%PAA Graft = 35%
60
38%
120
50%
B II. Ceric Ion Initiated Polymerication
PAA Density
Fresh Sample
3 Months Later
Activity (U/g PAA)
1000.0
800.0
600.0
400.0
200.0
0.0
2.3%
136%
360%
*Lipase from Candida rugosa (Sigma, EC 3.1.1.3, type VII)

FIG. 20

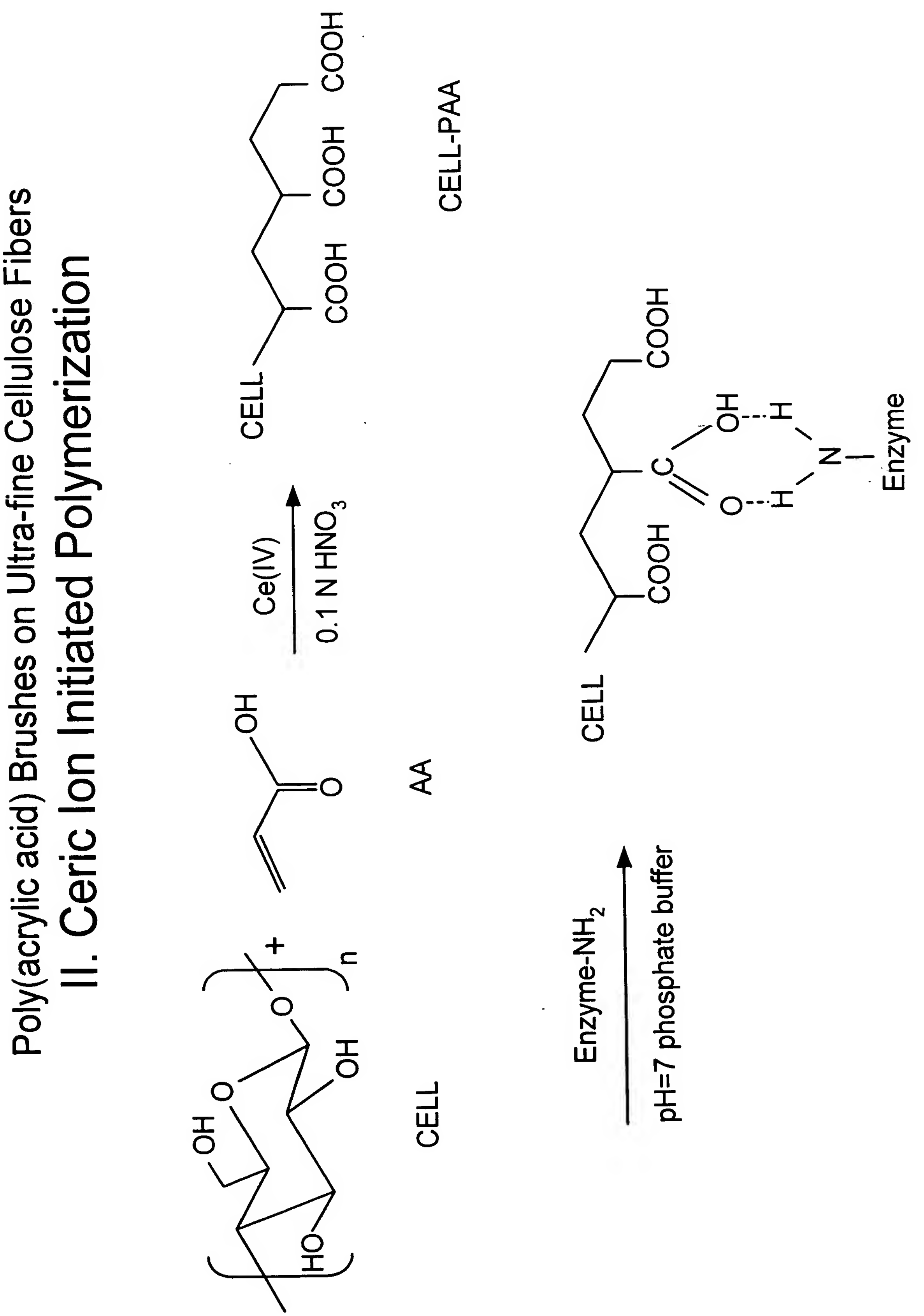
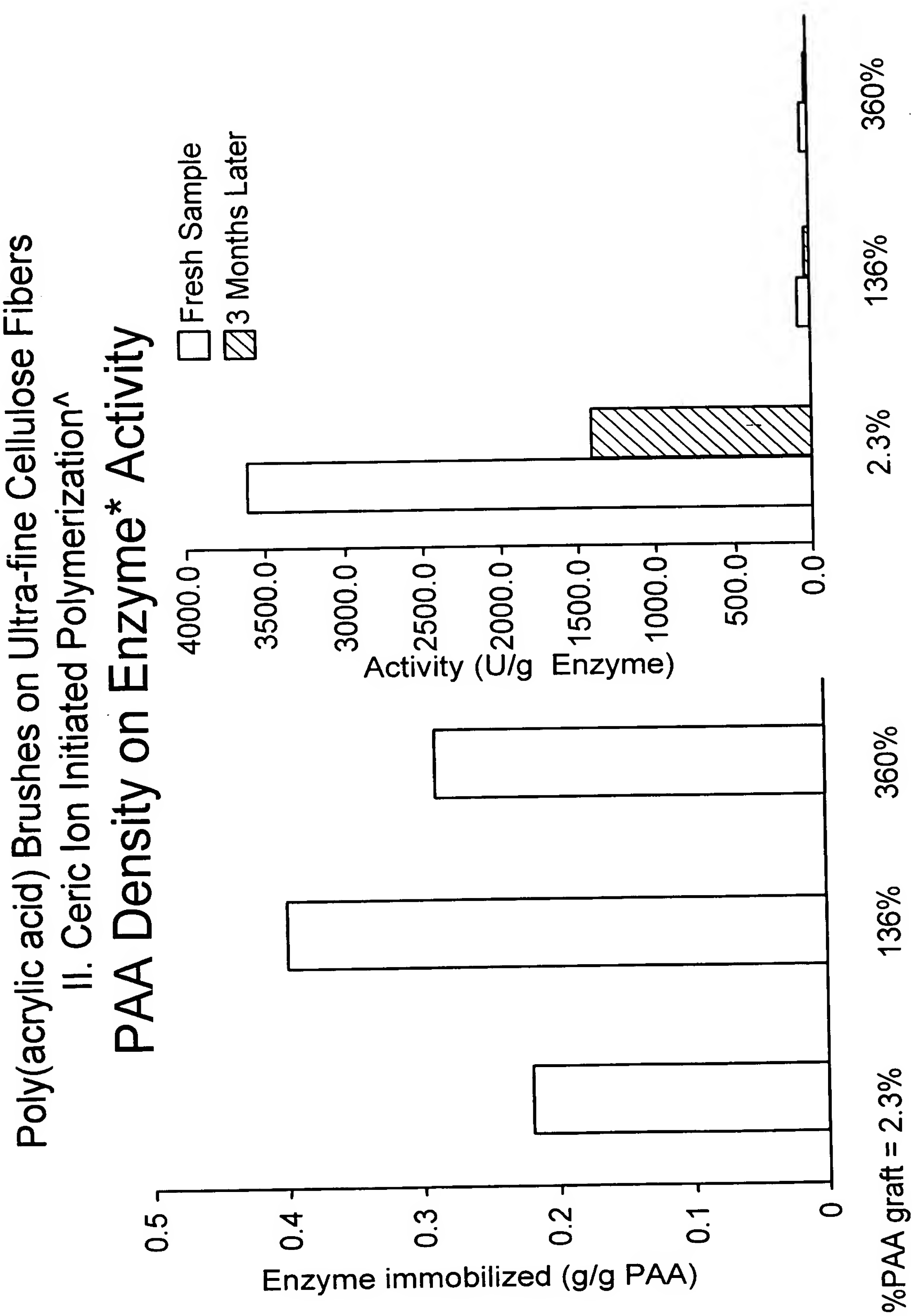

Poly(acrylic acid) Brushes on Ultra-fine Cellulose Fibers
II. Ceric Ion Initiated Polymerization
CELL
AA
Ce(IV)
0.1 N HNO$_3$
CELL-PAA
COOH
Enzyme-NH$_2$
pH=7 phosphate buffer
Enzyme

FIG. 21



^ Varying [AA] at const. 120 [AA]/[I]
* Lipase from Candida rugosa (Sigma, EC 3.1.1.3, type VII).

FIG. 22

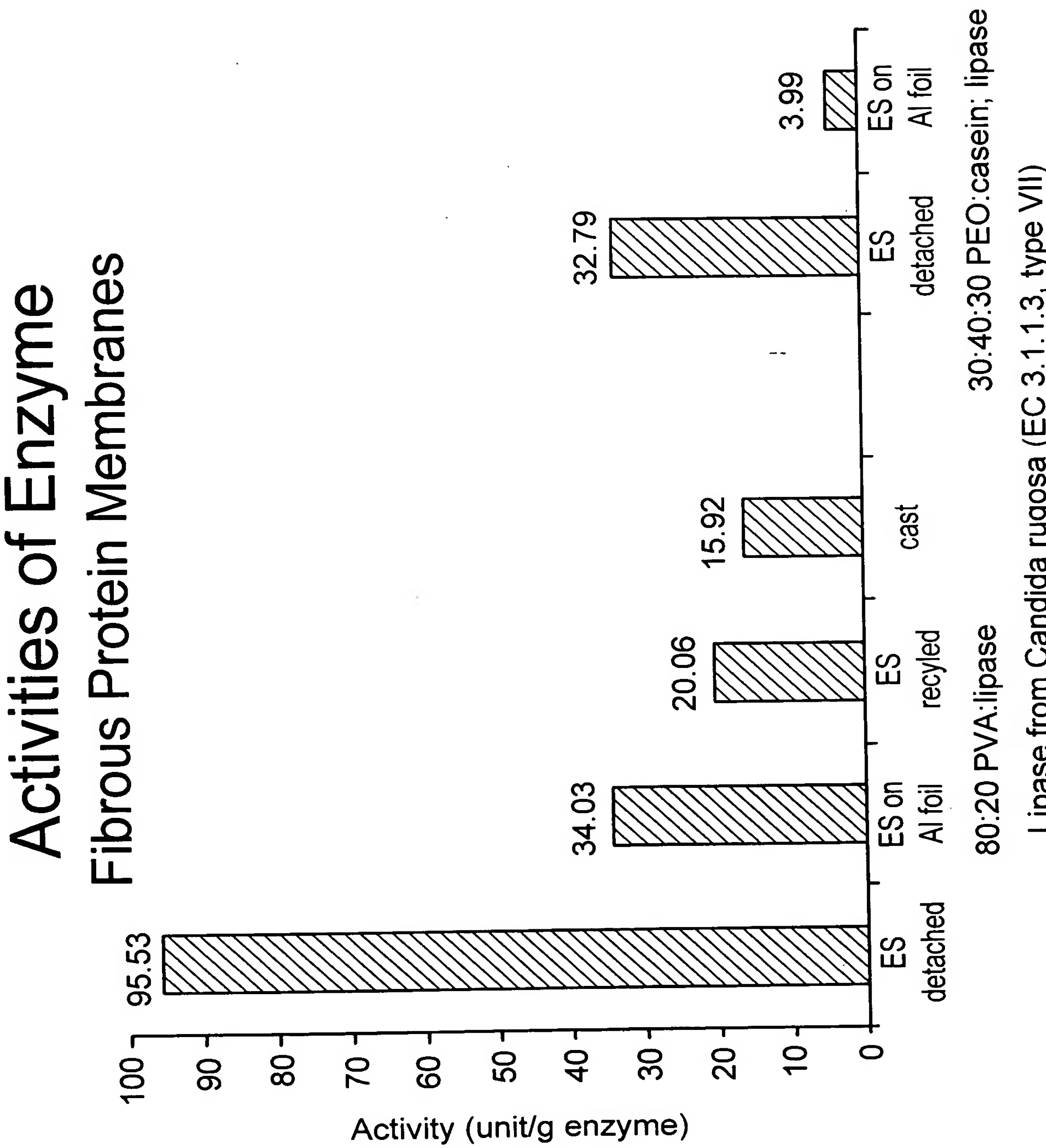

Activities of Enzyme
Fibrous Protein Membranes
Activity (unit/g enzyme)
0
10
20
30
40
50
60
70
80
90
100
95.53
34.03
20.06
15.92
32.79
3.99
ES detached
ES on Al foil
ES recyled
cast
ES detached
ES on Al foil
80:20 PVA:lipase
30:40:30 PEO:casein; lipase
Lipase from Candida rugosa (EC 3.1.1.3, type VII)

FIG. 23

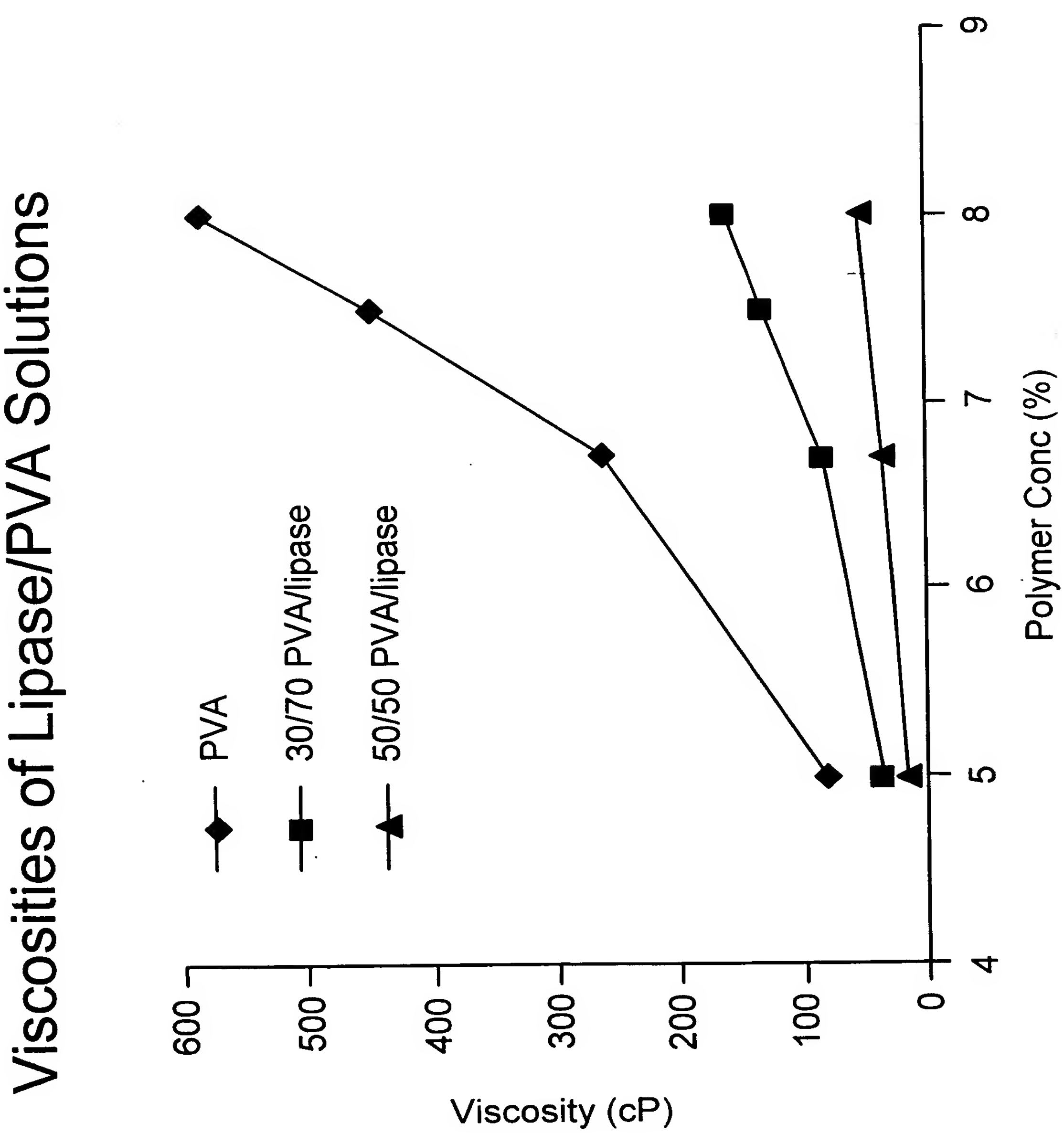

Viscosities of Lipase/PVA Solutions
PVA
30/70 PVA/lipase
50/50 PVA/lipase
Viscosity (cP)
0
100
200
300
400
500
600
Polymer Conc (%)
4
5
6
7
8
9

FIG. 24

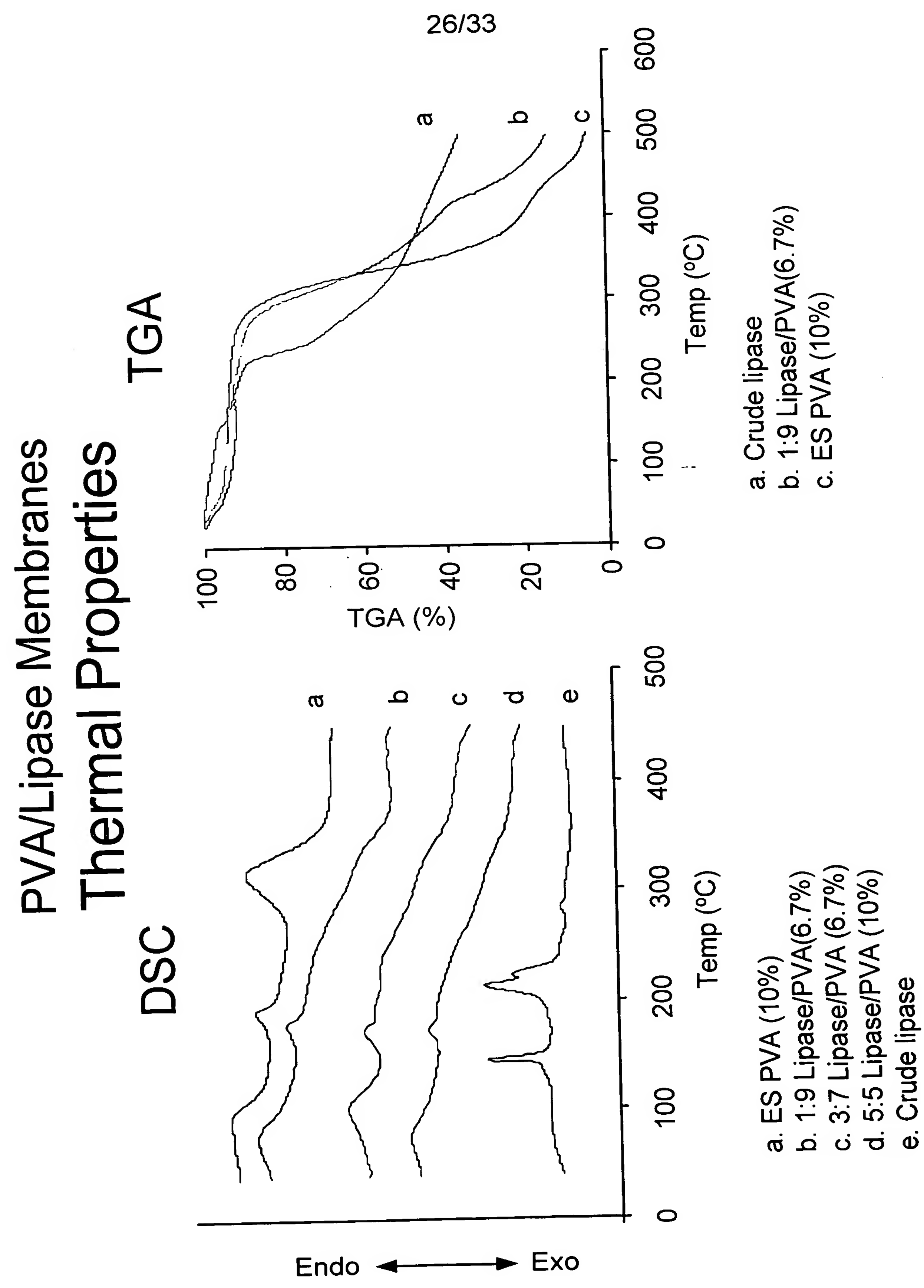
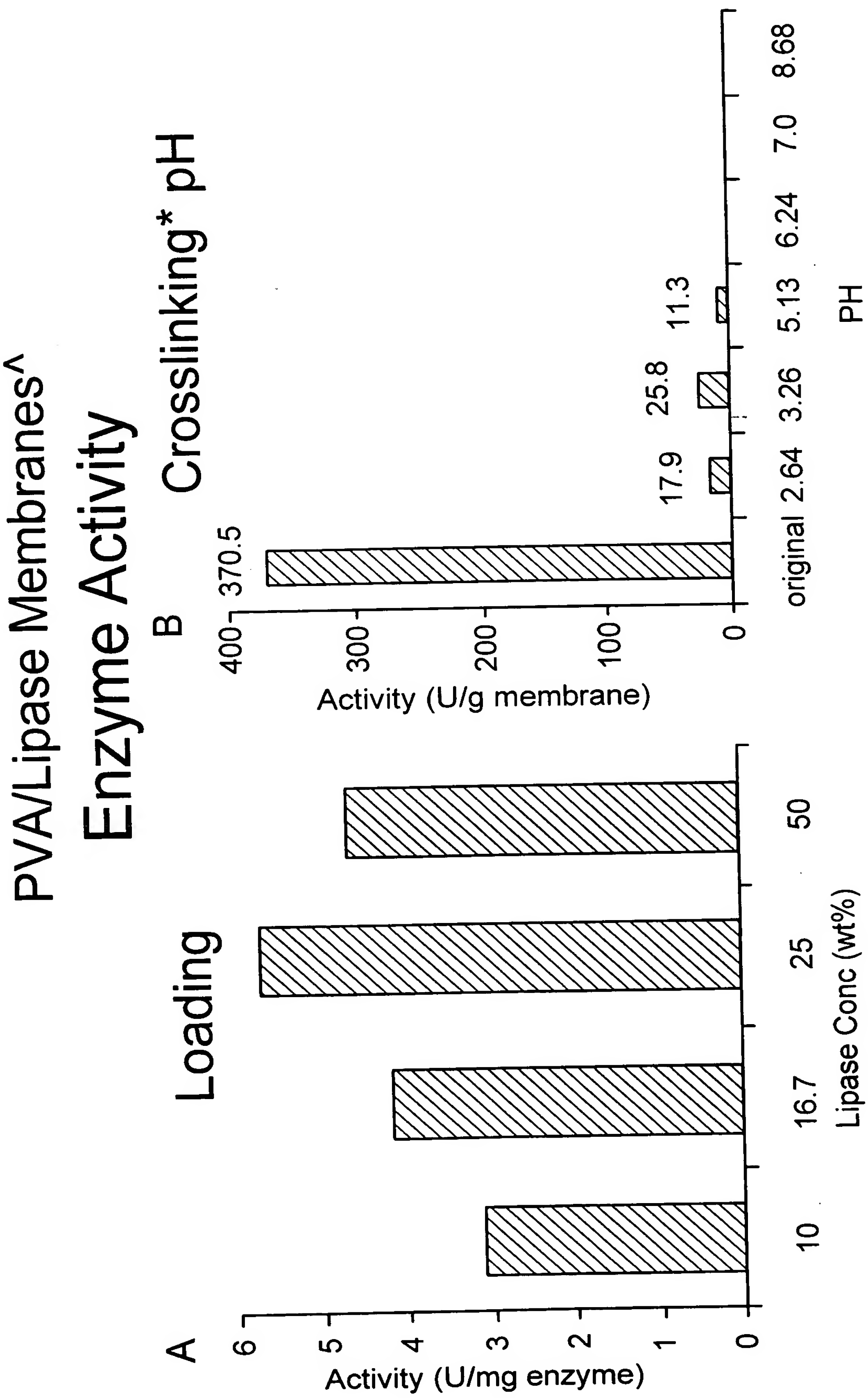

PVA/Lipase Membranes
Thermal Properties
DSC
TGA
Endo
Exo
0
100
200
300
400
500
Temp (°C)
a
b
c
d
e
a. ES PVA (10%)
b. 1:9 Lipase/PVA(6.7%)
c. 3:7 Lipase/PVA (6.7%)
d. 5:5 Lipase/PVA (10%)
e. Crude lipase
TGA (%)
100
80
60
40
20
0
600
a. Crude lipase
b. 1:9 Lipase/PVA(6.7%)
c. ES PVA (10%)

FIG. 25



^ 1:9 Lipase/PVA (6.7% aq. soln.
* 0.1 M GA in EtOH, 6 hr, ambient temperature

FIG. 26

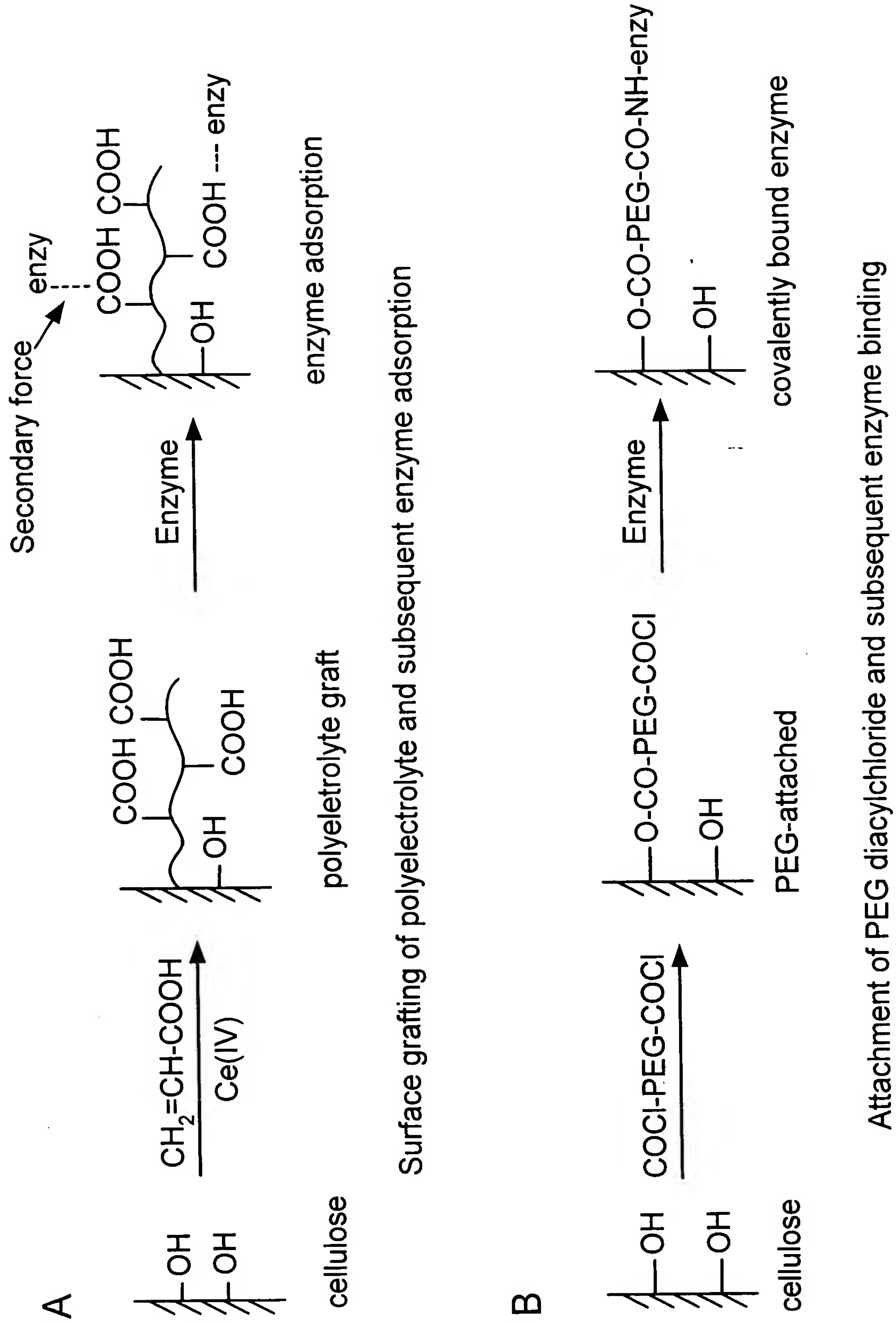
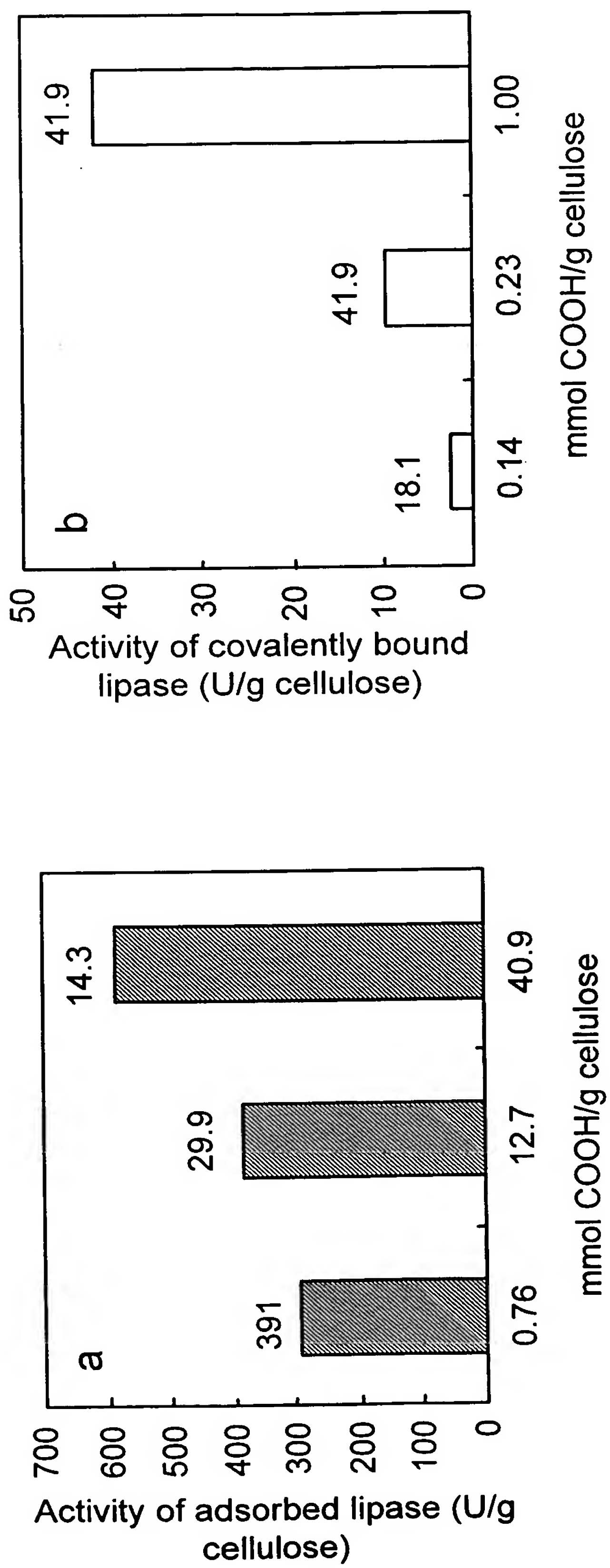
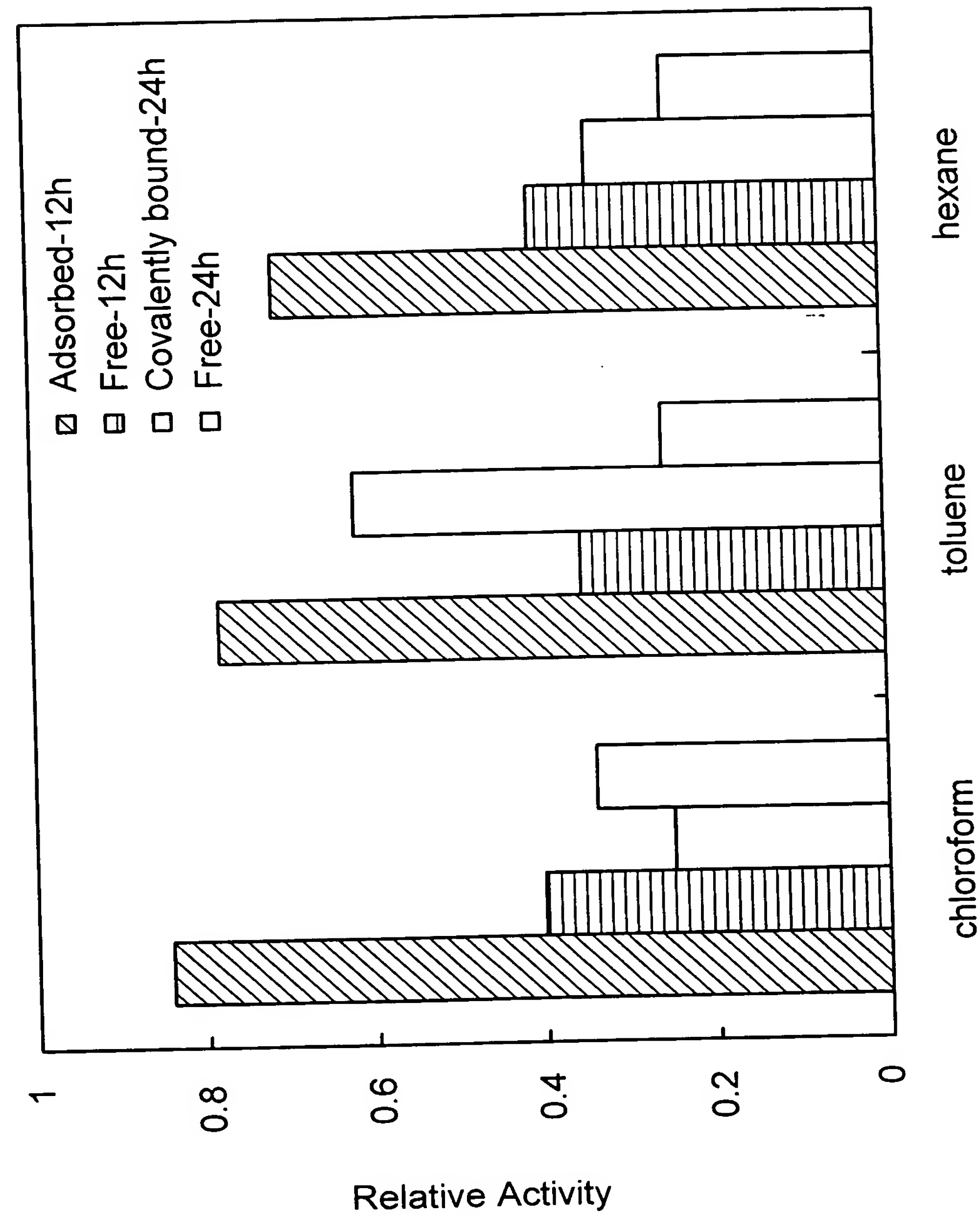

A
cellulose
CH2=CH-COOH
Ce(IV)
COOH COOH
COOH
OH
polyeletrolyte graft
Enzyme
Secondary force
enzy
COOH COOH
COOH --- enzy
OH
enzyme adsorption
Surface grafting of polyelectrolyte and subsequent enzyme adsorption
B
cellulose
COCl-PEG-COCl
O-CO-PEG-COCl
OH
PEG-attached
Enzyme
O-CO-PEG-CO-NH-enzy
OH
covalently bound enzyme
Attachment of PEG diacylchloride and subsequent enzyme binding

FIG. 27



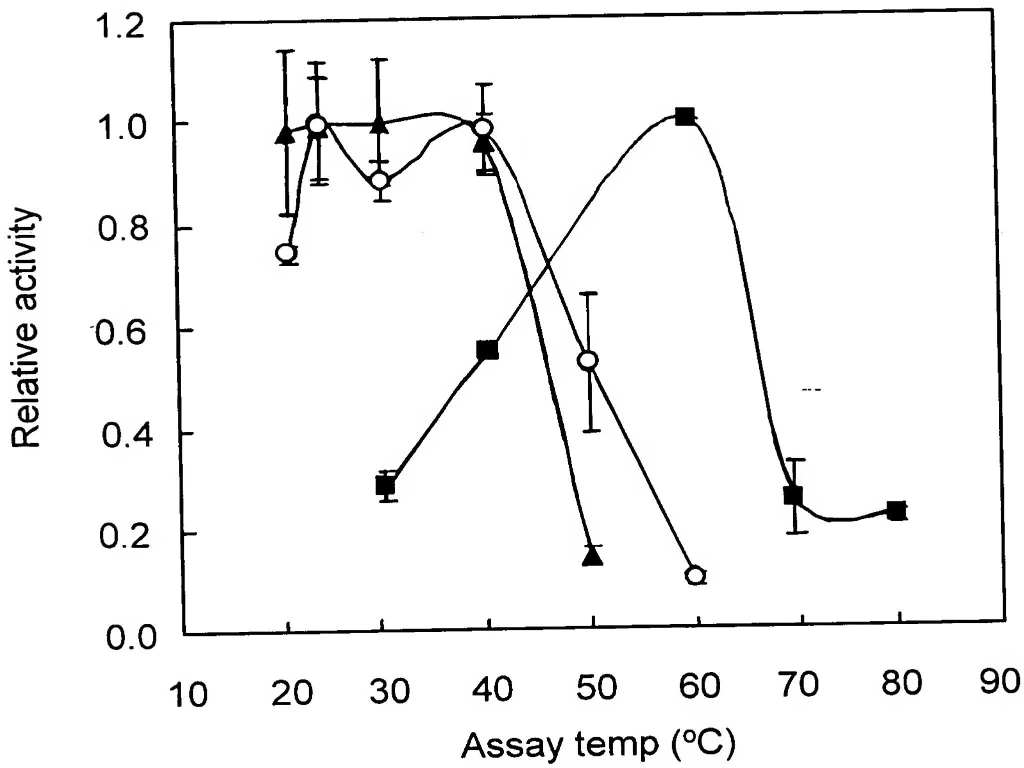
Effect of carboxylic acid quantity on the activity of bound lipase:
(a) adsorbed on PAA-grafted ([AA]/[Ce(IV)]=120;
(b) covalently bonded on PEG-grafted cellulose fibers

FIG. 28



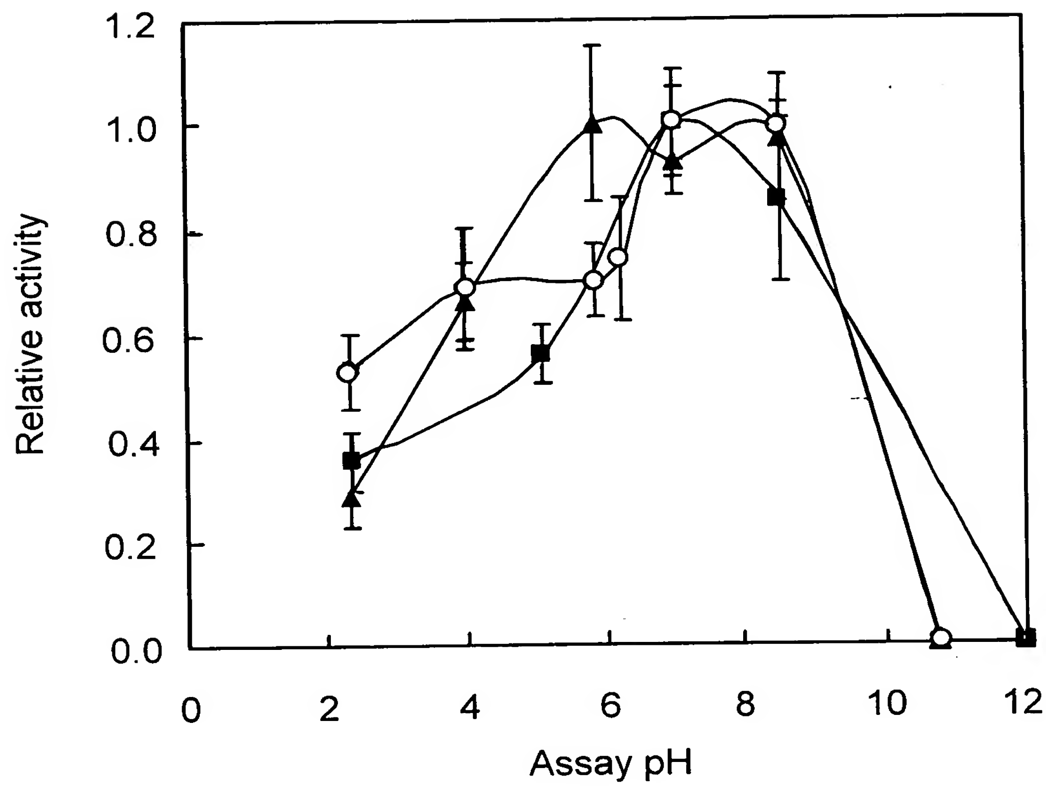
Relative activity of free and cellulose fiber bound lipase (pH=8.5, 30°C) following exposure to organic solvent

FIG. 29



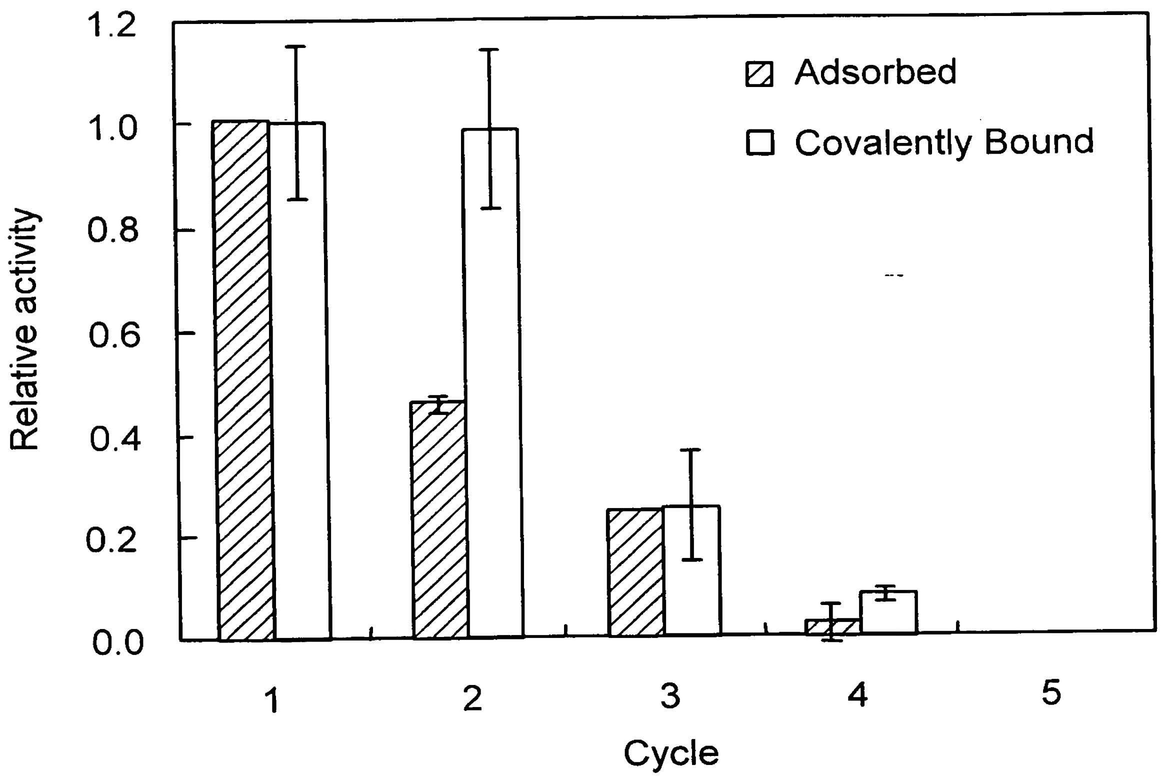
Relative activity (pH 8.5) of free lipase (o) and bound lipase on PAA-grafted (▲) and PEG-grafted (■) cellulose fibers at various temperatures

FIG. 30



Relative activity (30°C) of (o) free lipase and (▲) adsorbed lipase on PAA-grafted (■) covalently bound lipase on PEG grafted cellulose fibers under various assay pHs

FIG. 31



Cyclic activity (pH 8.5, 30°C) of bound lipase on cellulose fibers

FIG. 32